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# Serum vitamin A (retinol) reduction in broiler chicks on feed amended with *Fusarium proliferatum* culture material or fumonisin $B_1$ and moniliformin

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Fumonisins are mycotoxins produced by *Fusarium moniliforme,* a common fungal contaminant of corn worldwide,[10] and *F. proliferatum,* a closely related species.[1,10,19] The true incidence of *F. moniliforme* is difficult to determine because *F. proliferatum* has often been misidentified as *F. moniliforme.*[10,13] Certain strains of *F. proliferatum* also produce moniliformin, but isolates of *F. moniliforme* generally do not.[10] In many feeding studies, samples/diets were analyzed for fumonisin, but not moniliformin. It is possible that moniliformin and other toxic metabolites were present but not identified.

Contamination of corn by fumonisin at low levels (<1 μg/g) is common, but the actual concentration of fumonisin varies greatly with climatic conditions and geographic location.[17] Fungi capable of producing fumonisins can frequently be found in normal-appearing kernels, but corn screenings generally contain much higher concentrations of fumonisins and have been associated with episodes of animal toxicoses.[16]

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Fumonisin $B_1$ ($FB_1$) has been identified as the probable causative agent of equine leukoencephalomalacia, a fatal neurologic disease of horses and donkeys.[7,8,17,21] The toxin has been associated also with swine pulmonary edema and pancreatic and liver lesions.[3,4,14] Liver cancer has been observed in rats fed *F. moniliforme* culture material.[9]

Clinical signs, mortality, pathologic changes, reduced weight gains, serohematologic changes, and reduced immune response have been observed in broiler chicks given rations amended with *F. proliferatum* culture material that contained defined concentrations of $FB_1$, fumonisin $B_2$ ($FB_2$), and moniliformin or purified $FB_1$ and moniliformin given individually and in combination (T. Javed et al., unpublished).[5] Prominent gross lesions in affected birds included ascites, hydropericardium, hepatopathy, nephropathy, cardiomyopathy, pneumonitis, gizzard ulceration, and enlarged bursa of Fabricius with grumous material. Because the chicks given the mycotoxin-amended diets had clinical signs (ataxia, reduced weight gain, rough feathering, reduced lymphocyte viability, nephropathy) similar to those associated with vitamin A deficiency, serum vitamin A (retinol) concentrations in the affected chicks were determined, and dose–response effects were evaluated.

Two hundred twenty-eight male broiler chicks (Columbia × New Hampshire) were given feed amended with *F. proliferatum* M-5991 autoclaved culture material (CM) con-

**Table 1.** Serum vitamin A (retinol) in broiler chicks on feed amended with *F. proliferatum* culture material or with purified fumonisin $B_1$ and moniliformin.

| Treatment group | Concentration in feed (ppm) | | | No. days on feed[a] | No. birds sampled | Vitamin A (μg/ml)[b] |
|---|---|---|---|---|---|---|
| | $FB_1$ | $FB_2$ | Moniliformin | | | |
| T1[c] | 0 | 0 | 0 | 1–14 | 3 | 0.55 (0.44–0.64) |
| | | | | 1–28 | 2 | 0.63 (0.61–0.64) |
| T2 | 61 | 14 | 66 | 1–14 | 3 | 0.48 (0.37–0.55) |
| | | | | 1–21 | 3[d] | 0.78 (0.70–0.84) |
| | | | | 1–28 | 7 | 0.49 (0.36–0.58) |
| T3 | 193 | 38 | 193 | 1–28 | 1 | 0.26 |
| T4 | 546 | 98 | 367 | 1–5 | 2 | 0.10 (0.10) |
| PF1 | 0 | 0 | 0 | 1–28 | 5 | 0.58 (0.44–0.61) |
| PF2 | 61 | 14 | 66 | 7–28 | 4 | 0.55 (0.44–0.72) |
| PF3 | 193 | 38 | 193 | 7–14 | 4 | 0.11 (0.09–0.15) |
| PF4[e] | 546 | 98 | 367 | ... | ... | ... |
| R1 | 0 | 0 | 0 | 1–28 | 5 | 0.58 (0.44–0.61) |
| R2 | 61 | 14 | 66 | 21–28 | 2 | 0.61 (0.59–0.63) |
| R3 | 193 | 38 | 193 | 21–28 | 3 | 0.23 (0.21–0.27) |
| R4 | 546 | 98 | 367 | 21–28 | 4 | 0.15 (0.07–0.31) |
| P1[e] | 0 | 0 | 0 | ... | ... | ... |
| P2 | 125 | 0 | 0 | 1–14 | 4 | 0.44 (0.36–0.48) |
| P3 | 274 | 0 | 0 | 1–14 | 4 | 0.27 (0.19–0.40) |
| P4[e] | 0 | 0 | 27 | 1–14 | ... | ... |
| P5 | 0 | 0 | 154 | 1–14 | 4 | 0.43 (0.38–0.51) |
| P6 | 137 | 0 | 77 | 1–7 | 4 | 0.17 (0.07–0.25) |

[a] Number of days chicks were fed either control or amended diets; serum samples were taken on the last day indicated.

[b] Mean (range).

[c] Control chicks in T1 were used as controls for treatment groups T, PF and R.

[d] These 3 chicks were severely dehydrated; data have not been corrected for hemoconcentration.

[e] No serum samples were available.

taining combined $FB_1$ (61, 193, 546 ppm), $FB_2$ (14, 38, 98 ppm), and moniliformin (66, 193, 367 ppm) in 3 separate feeding trials (Table 1). Diets of treatment levels 2, 3, and 4 contained 5.1%, 16.0%, and 45.5% CM, respectively. The concentrations of $FB_1$, $FB_2$, and moniliformin in the various diets were determined by high-performance liquid chromatography (HPLC). Chicks received amended rations from day 1 (trial 1), day 7 (trial 2), and day 21 (trial 3) of age until day 28 of age. Purified $FB_1$ (125, 274 ppm) and moniliformin (27, 154 ppm) were given separately and in combination (137 ppm and 77 ppm, respectively) in a fourth feeding trial from day 1 until day 14 of age. Details of the experimental protocols, housing, and feeding management have been described.[5]

After chemical determinations (T. Javed, unpublished) had been performed on sera from chicks on the various amended diets, only 64 samples were available for further analysis. These samples were stored in screw-capped bottles[a] at −20 C and subsequently analyzed for retinol (Table 1). Retinol concentrations were determined using a modification of a method previously described.[18] One milliliter of serum was added to 1 ml of 100% ethyl alcohol for protein precipitation. The sample was vortexed, and 5 ml of HPLC-grade hexane was added; the sample was vortexed again and centrifuged at 3,500 rpm for 10 minutes. The upper hexane layer was removed and evaporated at 50 C under a nitrogen gas stream. The sample was redissolved in 200 μl of 100% ethyl alcohol and vortexed, and 10 μl was loaded onto an HPLC[b] column (5-μm particle size, 10 cm × 4.6 mm column).[c] The mobile phase was methanol : water (95:5) with a flow rate of 1.5 ml/minute. Retinol was detected at 325 nm with an ultraviolet diode array detector. Under these conditions, retinol had a retention time of 2.8 minutes. Standard and sample peaks were compared to determine the concentration of retinol present in the serum samples.

The various concentrations of $FB_1$, $FB_2$, and moniliformin in the amended diets produced well-defined dose–response effects in all groups of the 4 trials. Mortality, clinical signs, reduced weight gains, pathologic changes, immunologic and serohematologic changes were observed (T. Javed et al., unpublished).[5] Reduced weight gains may have been due to reduced feed intake; however, birds that died had feed in their digestive tracts, indicating that consumption continued through the early stages of toxicosis. The nutritionally balanced broiler ration[d] used contained 0.1% vitamin premix with a minimum of 1,000,000 IU vitamin A/lb.

Vitamin A (retinol) concentrations in sera from chicks on control and amended diets are presented in Table 1. There were dose-related reductions in serum concentrations of vitamin A in chicks given diets containing the 2 higher levels of *F. proliferatum* CM (193 ppm $FB_1$, 38 ppm $FB_2$, and 193 ppm moniliformin or 546 ppm $FB_1$, 98 ppm $FB_2$, and 367 ppm moniliformin). Compared with controls, there was a 75–86% reduction in mean serum retinol concentrations in the highest exposure groups and a 55–81% reduction in the medium exposure groups. Serum vitamin A in chicks given the lowest concentrations of mycotoxins (61 ppm $FB_1$, 14 ppm $FB_2$, 66 ppm moniliformin) were inconsistent and generally not different from controls. Depressed vitamin A concentrations were present in serum of chicks given diets amended individually with purified $FB_1$ (125, 274 ppm) or moniliformin (154 ppm) or with combined $FB_1$ (137 ppm) and moniliformin (77 ppm); however, these concentrations were higher than the levels present in the lowest CM diet.

These data, although preliminary in nature, clearly show reductions in serum retinol in chicks exposed to diets amended with high levels of fumonisin and moniliformin. The question remains, however, as to whether these reductions represent deficient retinol binding protein (RBP) production because of hepatic damage or whether they are the result of binding, inactivation, or destruction of vitamin A by these mycotoxins. Retinol is bound to RBP, which is synthesized by hepatocytes for the transport and delivery of retinol to target tissues of the body.[2,15] Both RBP synthesis and its secretion from hepatocytes to plasma is regulated by serum retinol concentrations. If the amount of RBP were reduced either by inactivation or decrease in synthesis and/or secretion, the result would probably be destruction or excessive renal excretion of vitamin A, either of which would lower the concentration of vitamin A in plasma or serum.

Histopathologic and ultrastructural changes in the livers of affected chicks indicate that endoplasmic reticulum (ER) and ribosomal damage occurs (T. Javed et al., unpublished).

Because the ER is the location of RBP-retinol complex formation, these lesions might relate to the depression of circulating retinol. Additionally, enzymes involved in the early part of the sphingomyelin biosynthetic pathway are located in the ER. Fumonisin affects sphingolipid biosynthesis by inhibiting the conversion of sphinganine to dihydroceramide.[11,20] The enzyme involved, sphinganine *N*-acyltransferase, is located on the cytoplasmic face of the ER.[6]

Characteristic clinical signs associated with vitamin A deficiency in chicks include ataxia, poor growth, and poor feather formation. Typical lesions of vitamin A deficiency are testicular degeneration, epithelial metaplasia, and distention of renal tubules and ureters with urates.[12] Affected chicks given the mycotoxin-amended diets in this study had many lesions that are compatible with vitamin A deficiency (T. Javed et al., unpublished.)[5]

*Addendum.* After this manuscript was submitted, the presence of the ionophore beauvericin was reported (Plattner RD, Nelson PE: 1994, Appl Environ Microbiol 60:3894–3896) in PE strains of *F. proliferatum,* which included strain M-5991 used in this study. Analysis of our stock CM indicated the following levels of toxins: 1,300 ppm $FB_1$, ~1,000 ppm moniliformin, and 1,100 ppm beauvericin.

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## Sources and manufacturers

a. Vacutainer Systems, Becton Dickinson, Rutherford, NJ.
b. 1090 Series HPLC, Hewlett-Packard, Wilmington, DE.
c. ODS Hypersil, Shandon Scientific, Cheshire, England.
d. Ration no. 4, Avian Research Center, University of Illinois, Urbana, IL.

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